Increased Rooting of 'Norton' Grape Cuttings Using Auxins and Gibberellin Biosynthesis Inhibitors

Kathryn Keeley¹, John E. Preece², and Bradley H. Taylor³

Department of Plant, Soil and General Agriculture, Southern Illinois University, Carbondale, IL 62901-4415

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Abstract. Hardwood and softwood cuttings of *Vitis aestivalis* Michx. 'Norton' were rooted under intermittent mist in a series of experiments using cuttings collected from two local vineyards. Hardwood cuttings treated in late March responded in a similar manner to KIBA and KNAA. Although there was little increase in the percentage rooting above 22.29 mM KIBA or 20.72 mM KNAA (5000 mg·L⁻¹ of either auxin), root number (but not root length) increased linearly on cuttings treated with up to 44.58 mM KIBA or 41.44 mM KNAA (10000 mg·L⁻¹ auxin). Cuttings treated with 10000 mg·L⁻¹ auxin produced up to 4 times more roots than the nontreated controls. The gibberellin biosynthesis inhibitors CCC and PAC had little effect on either hardwood cuttings or softwood cuttings that were harvested, treated and placed in the propagation bench in June. However, when softwood cuttings were collected in August, the most roots were found on cuttings treated with 50.6 mM CCC or 0.85 μ M PAC. Although all hardwood cuttings were collected at the same time and stored under refrigerated conditions, rooting percentage increased as storage time increased, especially on the nontreated control cuttings. When the cuttings were stored for the longest time (six weeks), KIBA no longer caused more roots per cutting. Chemical names used: potassium salt of indole-3-butyric acid (KIBA), potassium salt of α -naphthaleneacetic acid (KNAA), chlormequat chloride (CCC), paclobutrazol (PAC).

Vitis aestivalis is a grape species that is native to eastern North America. The cultivar 'Norton' is relatively winter hardy and disease resistant, and in Illinois, acreage planted with this cultivar is increasing (Dami, 2000). This trend will likely continue for the eastern United States as well because of the great quality and aging potential of 'Norton' wine (Morton, 1985). However, because of difficulties with rooting cuttings (Norton and Skirvin, 2001), planting stock is limited and costly.

'Norton' is extremely vigorous vegetatively (Wagner, 1978) and its cuttings root poorly. These two characteristics are typical of plants that produce relatively high levels of gibberellins. Although there are no reports on the hormonal content of 'Norton', there is a general inverse relationship between gibberellin content in cuttings and their ability to form adventitious roots and when gibberellins are applied to cuttings rooting is inhibited (Hansen, 1988). For example, gibberellic acid completely inhibited rooting of *V. vinifera* L. 'Chaush' cuttings (Eris and Celik, 1981). Soaking grape cuttings in water for 24 h increased rooting from 70% to 83% of an easy-to-root clonal rootstock '5BB' (*V. berlandieri* Planch. $\times$ *V. riparia* Michx.) and from 18% to 38% for a difficult-to-root clonal rootstock '140 Ruggeri' (*V. berlandieri* $\times$ *V.*

rupestris Scheele.) (Bartolini et al., 1986). This increase in rooting was attributed, in part, to leaching of gibberellin-like substances out of the cuttings. Furthermore, Kracke et al. (1981) found that an easy-to-root grape clonal rootstock 'Kober 5 BB' (*V. berlandieri* $\times$ *V. riparia*) had a substantially lower endogenous gibberellin content than '140 Ruggeri'.

Plantings of 'Norton' are limited because cuttings are difficult to root. Propagators often have 30% or less rooting of 'Norton' cuttings (personal communications) and 40% to 50% rooting is considered to be excellent (Norton and Skirvin, 2001). Therefore the objective of this study was to test concentrations of the auxins, potassium salt of indole-3-butyric acid (KIBA) and the potassium salt of α -naphthaleneacetic acid (KNAA), and the gibberellin biosynthesis inhibitors chlormequat chloride (CCC) and paclobutrazol (PAC) in an attempt to increase rooting of hardwood and softwood cuttings of 'Norton'.

Materials and Methods

Plant materials. Dormant, three node, 20 $\pm$ 3 cm long hardwood cuttings with 15–100 mm long internodes and 3–7 mm diameter of *Vitis aestivalis* 'Norton' were taken from 3-year-old vines at Mockingbird Farms, Makanda, Ill. and 8-year old vines at Owl Creek Vineyards, Cobden, Ill., on 26 and 27 Feb. 1999. The cuttings were stored in closed 125-L, 0.02-mm-thick black polyethylene garbage bags with two damp paper towels at 5 °C until used. Actively growing 15 $\pm$ 2 cm long softwood cuttings with 15–60

mm long internodes and 3 to 7 mm diameter were collected at Mockingbird Farms 26 June 1999 and Owl Creek Vineyards 2 Aug. 1999. All foliage was removed, except the leaf from the most distal node. If the leaf at this node was >8 cm long, the terminal half of the leaf was excised.

All cuttings were disinfested by a 5 second immersion in a hydrogen dioxide and peroxyacetic acid solution (ZeroTol; Biosafe Systems, Glastonbury, Conn.), at the rate of 9.5 mL of ZeroTol per liter of water]. Following immersion in the ZeroTol solution, fresh cuts were made on each end of the hardwood cuttings (leaving all three nodes) and directly below the bottom node for the softwood cuttings. Then, the basal 10 mm of all cuttings were dipped in treatment solutions (all prepared using deionized water) for 30 s unless otherwise stated. The bottom two nodes of cuttings were placed within the medium, leaving only the top node uncovered.

Hardwood cuttings were rooted in a moist 1 vermiculite : 1 perlite (by volume) medium in plastic Rootainers (Hummert Intl., Earth City, Mo.) each with a cell volume of 90 cm³. Softwood cuttings were rooted in the same medium in standard 10-cm pots.

Environmental. Hardwood cuttings were rooted on a greenhouse bench with bottom heat at 30 °C and mist set on a timer at 6 s every 6 min for 13 daylight hours per day and with air temperature maintained at 21 °C. Because of the hot summer conditions, no supplemental bottom heat was used for the softwood cuttings, which were also placed under intermittent mist at 3 s every 3 min with an air temperature of 30 °C day/24 °C night. Cuttings were drenched with etridiazole and thiophanate-methyl (7.5 g Banrot 40% of WP per liter of water) for control of fungal pathogens if there was any sign of browning of the roots or stems during the rooting process.

Hardwood cuttings treated with KIBA and KNAA. After 28 d of cold storage, on 26 Mar. 1999, hardwood cuttings were treated with 0, 11.145, 22.29, 33.435, or 44.58 mM KIBA (0, 2500, 5000, 7500, or 10000 mg of KIBA per liter of water); 0, 10.36, 20.72, 31.08, or 41.44 mM KNAA (0, 2500, 5000, 7500 or 10000 mg of KNAA per liter of water); or a combination of 11.124 + 10.36, 11.124 + 20.72, 22.29 + 10.36, or 22.29 + 20.72 mM KIBA + KNAA.

Timing of KIBA soaks on hardwood cuttings. After 41 d of cold storage, on 7 Apr. 1999, hardwood cuttings were treated with 0, 1.115, 2.229, 11.145, or 22.29 mM KIBA for either 30 s, or 24 h. This resulted in a 5 $\times$ 2 factorial combination of auxin concentrations and duration of treatments.

Hardwood cuttings treated with gibberellin biosynthesis inhibitors: CCC, PAC, and the auxin KIBA. After 48 d of cold storage, on 14 Apr. 1999, hardwood cuttings were treated 0, 3.16, 6.33, 12.65, 25.3, or 50.6 mM CCC (0, 500, 1000, 2000, 4000, or 8000 mg CCC per liter of water) or 0, 1.7, 3.4, 6.8, 13.6, or 27.2 μ M PAC (0, 0.5, 1.0, 2.0, 4.0, or 8.0 mg of PAC per liter of water). Half of the cuttings in each treatment were treated with 22.29 mM KIBA and the other half received no auxin treatment.

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¹Former Graduate Student.

²Professor, to whom reprint requests should be addressed.

³Associate Professor.

Softwood cuttings treated with gibberellin biosynthesis inhibitors: CCC, PAC, and the auxin KIBA. On 26 June 1999, softwood cuttings were collected at Mockingbird Farms, and on 2 Aug. 1999, cuttings were collected from Owl Creek Vineyards. Cuttings were treated 0, 1.58, 3.16, 6.33, 12.65, 25.3, or 50.6 mM CCC or 0, 0.85, 1.7, 3.4, 6.8, 13.6, or 27.2 μ M PAC. Half of the cuttings in each treatment were treated with 4.458 mM KIBA (1000 mg of KIBA per liter of water) and the other half received no auxin treatment.

Experimental design and statistical analysis. In each experiment, cuttings were arranged in a randomized complete-block design with 10 blocks per run and one cutting per treatment per block, with blocking according to cutting diameter and internode length. Each experiment was conducted concurrently from the mature vines at Owl Creek Vineyards for one run and cuttings from the recently established vines at Mockingbird Farms for the other run. Data were collected on root number per cutting, root length and percentage rooting within treatment and analyzed using the general linear model procedures (SAS Inst., 1996) to generate analysis of variance (ANOVA). Runs were tested for homogeneity and were separated if heterogeneous. Main effects and interactions for a fixed effects model were tested using pooled run and block(run) interactions. Where appropriate, data were transformed using the $(y + 0.5)^{0.5}$ or $\log(y + 1.0)$ to normalize data (Steel and Torrie, 1980). Level of significance, contrasts where appropriate and *t* tests for paired comparisons are presented in tables and figure legends.

Results

Hardwood cuttings treated with KIBA and KNAA. Because the cuttings responded in a

similar manner to both auxins (according to F-test), the responses were averaged across the KIBA and KNAA treatments. Increasing the auxin concentration caused a linear response for the number of roots that formed on each cutting; however, according to *t* test, auxin concentrations >20.72 mM did not significantly increase root number more than the 20.72 or 22.29 mM concentration (Fig. 1). According to F test, rooting percentage and root length were not significantly affected by auxin concentration.

Timing of KIBA soaks on hardwood cuttings. Soaking duration in KIBA solution (30 s or 24 h) did not significantly affect the number, length or percent rooting according to F-test (tabular data not shown). Again, root number, (but not length) increased significantly ($P < 0.05$) from 2.0 roots per control cutting to 4.7 roots as concentration increased to the maximum of 22.29 mM.

Hardwood cuttings treated with gibberellin biosynthesis inhibitors: CCC, PAC, and the auxin KIBA. These hardwood cuttings had been in storage for ~6 weeks prior to treating and placing under mist. Controls rooted 65% (Owl Creek) or 90% (Mockingbird Farms), which indicated that the amount of time in storage increased rooting to such an extent that treatments made little difference. The gibberellin biosynthesis inhibitors had no significant effect on number of adventitious roots produced by the hardwood cuttings or percent rooting (data not shown).

Softwood cuttings treated with gibberellin biosynthesis inhibitors: CCC, PAC, and the auxin KIBA. There was a significant ($P < 0.05$) 3-way interaction among gibberellin biosynthesis inhibitors, concentration, and KIBA on the softwood cuttings taken in June. The softwood controls rooted at 90% and had 5

to 7 roots per cutting with a root length of 13 to 22 mm (Table 1). The effects of auxin and gibberellin biosynthesis inhibitors on root number and root length varied with concentration.

For the cuttings taken in August, there was a significant interaction between gibberellin biosynthesis inhibitors and concentration for both root number and length. Highest root numbers were on cuttings treated with 50.6 mM CCC or 0.85 μ M PAC (Fig. 2). Cuttings with either of these treatments produced more than double the number of roots compared to the control cuttings. This indicates that PAC is thousands of times more active than CCC for stimulating root initiation in softwood 'Norton' grapevine cuttings, especially when taken at a less optimal time (August) for softwood cuttings.

Timing. With the hardwood cutting experiments, the control cuttings rooted better with each successive experiment. Although the experiments were not originally designed to be compared over time, there were two treatments that each had in common: the control and 22.29 mM KIBA. A significant ($P < 0.05$) difference was noted for root number as duration of cold storage of cuttings increased (Table 2). Cuttings

Table 1. Effect of growth retardant, concentration and KIBA on mean root number, mean root length, and percent rooting of softwood 'Norton' grape cuttings² taken in June 1999.

Retardant concentration	KIBA (mM) ¹	Root no.	Root length (mm)	Rooting (%)
CCC				
0	0	5.0	13.1	90
	4.458	8.8	24.2	90
1.58	0	8.3	23.0	100
	4.458	5.1	19.0	90
3.16	0	5.4	30.6	90
	4.458	4.8	11.0	80
6.33	0	6.7	15.7	90
	4.458	3.4	7.5	70
12.65	0	5.2	18.2	90
	4.458	4.5	18.7	90
25.30	0	7.4	19.7	80
	4.458	4.8	15.2	80
50.60	0	4.3	19.2	80
	4.458	6.8	20.8	90
PAC (μ M)				
0	0	6.8	21.8	90
	4.458	6.9	19.4	90
0.85	0	7.1	15.8	90
	4.458	4.7	20.0	90
1.7	0	4.8	16.4	90
	4.458	8.1	14.4	80
3.4	0	4.3	13.2	80
	4.458	7.7	17.9	80
6.8	0	5.0	13.3	70
	4.458	5.2	15.4	70
13.6	0	3.3	7.3	80
	4.458	7.5	26.5	90
27.2	0	7.4	28.7	100
	4.458	5.1	15.2	90
Significance		*	*	
5% <i>t</i> test ³		3.8	13.8	

²These calculations are based on the mean of 10 cuttings from Mockingbird Farms.

³For statistical analysis, data were transformed using the $\log(y + 1.0)$ transformation. Nontransformed means are presented.

⁴*t* test for paired comparisons.

⁵Significant at the 5% level according to F test with 6 and 243 df

Fig. 1. The effect of auxin concentration on mean root number, mean root length, and percent rooting of hardwood cuttings of 'Norton' grape planted 26 Mar. 1999. For statistical analysis, root number, and root length data were transformed using the $(y + 0.5)^{0.5}$ transformation, nontransformed means are presented. The number of roots per cutting was significant at the 1% level according to the F test with 4 and 171 df. According to a single degree of freedom contrast, the effect of increasing concentration of auxin was significantly linear at the 1% level according to F test with 1 and 171 df. The 5% and 1% *t* test values for root number are 2.5 and 3.3, respectively. The root length and rooting percentage data were not significant. Each mean is based on 40 cuttings averaged across KIBA and KNAA. The auxin concentrations, control, low, middle, higher, and highest represent 0, 2500, 5000, 7500, and 10000 mg·L⁻¹ auxin, which are equivalent to 0, 11.145, 22.29, 33.435, or 44.58 mM KIBA and 0, 10.36, 20.72, 31.08, or 41.44 mM KNAA.

Fig. 2. The effect of (A) chlormequat chloride (CCC) and (B) paclobutrazol (PAC) concentrations on mean root number, mean root length and percent rooting of softwood 'Norton' grape cuttings taken in Aug. 1999. For statistical analysis, root number and root length data were transformed using the $\log(y + 1.0)$ transformation, nontransformed means are presented. Root number was significant at the 1% level, and root length was significant at the 5% level according to F test with 6 and 243 df. The 5% and 1% *t* test values for root number are 2.1 and 2.7 and for root length are 7.0 and 9.3, respectively. The percentage data were not significant. Each mean is based on 20 cuttings averaged across two auxin concentrations.

Table 2. Effect of date 'Norton' grape cuttings were prepared, treated, and placed under intermittent mist on mean root number, mean root length, and percent rooting² of hardwood cuttings.

Experiment date	Days in storage	Root no.		Root length		Rooting (%)	
		KIBA 0	mm 22.29	KIBA 0	mm 22.29	KIBA 0	mm 22.29
26 Mar. 1999	(28)	0.9	5.5	18.9	23.5	30	55
7 Apr. 1999	(41)	2.0	5.5	14.9	20.1	65	55
14 Apr. 1999	(48)	1.7	1.4	24.0	19.0	77.5	72.5
Significance		*		NS		NS	
5% <i>t</i> test ¹		1.5					

¹Calculations based on mean of 20 cuttings (28 d and 41 d) and 40 cuttings (48 d).

²*t* test for paired comparison.

NS, *Nonsignificant or significant at the 5% level or nonsignificant according to F test with 2 and 133

that were in cold storage for short amounts of time produced significantly more roots when treated with KIBA than when not treated with auxin. As time in cold storage increased to 48 d, the addition of KIBA had no effect. There was no significant effect of storage time on root length or rooting percentage.

Discussion

Rooting was improved during early spring with application of auxin to 'Norton' hardwood cuttings. This effect was on hardwood cuttings that had been in cold storage for only 28 d. However, hard-to-root grapevines are frequently subjected to cold storage, pre-callusing, and application of plant growth regulators to enhance rooting (Warmund et al., 1986). According to Alley (1979), applications of IBA treatments on hard-to-root rootstock cuttings

such as *Vitis champini* 'Salt Creek' and 'Dog Ridge' improved overall rooting.

We noted a greater effect of the gibberellin biosynthesis inhibitors on root number and length on softwood cuttings than on hardwood cuttings. However, results for initiating rooting with the application of gibberellin biosynthesis inhibitors were inconsistent, especially when the softwood cuttings were placed in the mist bench at the optimum time (June). Gibberellin biosynthesis inhibitors were most useful in rooting softwood cuttings taken at a suboptimal time for rooting (August). Various gibberellin biosynthesis inhibitors have been shown to promote rooting (Davis and Sankhla, 1988), and when CCC was applied to *V. vinifera* 'Chauth' cuttings, it increased the root fresh weight (Eris and Celik, 1981).

Long-term cold storage (minimum of 40 d) may increase rooting of grape hardwood

cuttings. Southeastern Missouri commercial wine grape propagators indicated that cuttings placed into a rooting medium during late spring tended to root better than cuttings placed in the medium earlier that same year (personal communication), which agrees with our findings on hardwood cuttings. Further research on time of propagation and length of hardwood cutting storage is needed to improve the horticultural application of our results.

It has been noted that 'Norton' is vegetatively vigorous (Wagner, 1978) and the most undesirable characteristic of 'Norton' vines to propagators is the poor rooting of cuttings (Tarara and Hellman, 1991). Generally, easy-to-root grapevines are propagated by dormant hardwood cuttings without the use of special rooting techniques. 'Norton' hardwood cuttings can be rooted at a high percentage with applications of auxin and/or cold storage prior to rooting. Softwood cuttings will root in a high percentage if they are taken during the early summer when the vines are actively growing.

Literature Cited

- Alley, C.J. 1979. Grapevine propagation/VI. Rooting of cuttings: effects of indolebutyric acid (IBA) and refrigeration on rooting. *Amer. J. Enol. & Viticult.* 30(1):28-32.
- Bartolini, G., M.A. Toponi, and L. Santini. 1986. Endogenous GA-like substances in dipping waters of cuttings of two *Vitis* rootstocks. *Amer. J. Enol. & Viticult.* 37:1-6.
- Dami, I. 2000. 1999 grape growers and vintners survey, p. 1-8. In: *Proc. Illinois Grape Growers and Vintners Assn. Annu. Conf.*, Springfield, Ill.
- Davis, T.D. and N. Sankhla. 1988. Effect of shoot growth retardants and inhibitors on adventitious rooting, p. 174-184. In: T.D. Davis, B.E. Haissig, and N. Sankhla (eds.). *Adventitious root formation in cuttings*. Dioscorides Press, Portland, Ore.
- Eris, A. and H. Celik. 1981. Effects of some plant growth regulators on bud burst and rooting of *Vitis vinifera* L. cv. Chauth cuttings. *Amer. J. Enol. Viticult.* 22:122-124.
- Hansen, J. 1988. Influence of gibberellins on adventitious root formation, p. 162-173. In: T.D. Davis, B.E. Haissig, and N. Sankhla (eds.). *Adventitious root formation in cuttings*. Dioscorides Press, Portland, Ore.
- Kracke, H., G. Cristofori, and B. Marangoni. 1981. Hormonal changes during the rooting of hardwood cuttings of grapevine rootstocks. *Amer. J. Enol. & Viticult.* 32:135-137.
- Morton, L.T. 1985. *Winegrowing in Eastern America*. Cornell Univ. Press, Ithaca, N.Y.
- Norton, M.A. and R.M. Skirvin. 2001. Micropropagation of 'Norton' winegrape. *HortTechnology* 11: 206-208.
- SAS Institute. 1996. The SAS system. Release 6.12. SAS Inst., Cary, N.C.
- Steel, R.G.D. and J.H. Torrie. 1980. *Principles and procedures of statistics a biometrical approach*, 2nd ed. McGraw-Hill, New York.
- Tarara, J.M., and E.W. Hellman. 1991. Norton and Cynthiana premium native wine grapes. *Fruit Var. J.* 45(2):66-69.
- Wagner, P.M. 1978. *A wine-growers guide*. Revised ed. Alfred A. Knopf, New York.
- Warmund, M.R., C.J. Starbuck, and L. Lockshin. 1986. Growth, cold hardiness, and carbohydrate content of Vidal blanc grapevines propagated by hardwood and softwood cuttings. *Amer. J. Enol. & Viticult.* 37(3):215-219.